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COMBINED EFFECTS OF SHRIMP SIZE AND DIETARY PROTEIN SOURCE ON THE GROWTH OF *Penaeus setiferus* AND *P. vannamei*

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ABSTRACT

Three size classes of juvenile *Penaeus setiferus* (average wet weight = 0.08, 0.99, and 3.82 g) and *P. vannamei* (average wet weight = 0.12, 1.39, and 4.72 g) were kept 3 weeks in indoor tanks at 28°C, and each of 3 isonitrogenous (28% protein) experimental diets were fed to each size class. Shrimp meal of a basal experimental diet was replaced by fish protein hydrolysate, soybean meal, and cotton seed meal to achieve animal:plant (A:P) protein ratios of 1:1.5, 0.9:1, and 1.5:1 in the 3 diets. *Penaeus vannamei* grew faster than *P. setiferus* given the same diets. For both species, A:P ratios of food improved growth for all 3 size categories of shrimp. This effect was significantly greater in the small animals than in the medium or larger-sized ones. Thus, the quality of diet needed to achieve maximum growth appears to be size dependent, smaller shrimp being more dependent than larger ones on animal protein. Results also indicated that the two larger size classes of *P. setiferus* require higher animal protein in their feed for maximum growth than do similar size classes of *P. vannamei*.

INTRODUCTION

Tests in feeding fry, fingerling, and adult fish have shown that gross protein requirements are highest in smallest feeding fry and decrease as fish size increases. To grow at a maximum rate, salmonid fry must have a diet with 50% balanced protein; at 6-8 weeks this requirement is decreased to about 40% and is further reduced to 35% for adult fish (Halver 1978). In their reviews of shrimp biology and nutrition, both New (1976) and Wickins (1976) stated that the nutritional requirements of shrimp are likely to change with age (size). Balazs et al. (1974) in a feed trial with *Macrobrachium rosenbergii* found that a faster growth rate was achieved with higher dietary protein levels during the first two measurement periods (0-119 days), but the converse was true in the third period (119-175 days). Colvin and Brand (1977) reported that the dietary protein requirement of early post-larval penaeid shrimp exceeds 40% crude protein, but decreases to less than 30% in the later life-cycle stages.

There have been various attempts to elucidate size-dependent nutritional requirements. In most species of fish studied, size has been found to be independent of assimilation efficiency of dietary total energy, protein, and lipid (Menzel 1960; Pandian 1967; Elliot 1976; Targett 1979). In contrast, Fenucci et al. (1982) demonstrated an inverse relationship between *P. stylirostris* size and both protein and carbohydrate assimilation, and suggested the presence of a more developed enzymatic system in larger shrimp. However, no direct relationship between protein assimilation and shrimp growth has been found (Fenucci et al. 1982; Colvin 1976). Although the need for raw materials (especially protein) for body synthesis is important during growth (Hysmith et al. 1972), the amount of required protein used for body synthesis decreases with more used for metabolic activity as animals become larger.

Quantitative nutritional requirements of shrimp have been closely linked to their qualitative needs (New 1976). With inferior protein quality, optimal shrimp growth can only be achieved at protein levels greater than those required with a higher quality protein source. Many studies have focused on quantitative requirements for protein and other ingredients as related to shrimp size. However, the effect of dietary protein type (or quality) on shrimp of different sizes has rarely been examined. New (1976), extrapolating from the study by Balazs and Ross (1976), suggests that plant protein is more suitable for older juvenile *Macrobrachium rosenbergii* than for young juveniles and that the converse is true for dietary animal protein. In order to test this hypothesis for penaeids, the present study compares the effects of three isonitrogenous diets with protein sources of different quantity and quality on the growth of three size classes of *Penaeus setiferus* and *P. vannamei*, two species of commercially important shrimp.

MATERIALS AND METHODS

Experimental glass aquaria with 60-liter capacity were prepared with undergravel filter beds of oyster shell and sand and filled with 50 liters of UV-sterilized natural seawater. De-ionized water was used to adjust salinity to 26-28 ppt and no seawater was changed or added throughout the experiment. The aquaria were located in walk-in incubators controlled at 28±1°C and a constant photoperiod of 12 hours light and 12 hours dark.

Three size classes of *Penaeus setiferus* and *P. vannamei* (approximately 0.1 g, 1-1.5 g, and 4-5 g corresponding to approximately 4, 6-7, and 10-12 weeks, respectively, after hatching) were used in the study (Table 1). These sizes were selected to permit the rapid growth characteristic of smaller juvenile shrimp, while avoiding growth-restricting effects caused by the relatively limited carrying capacity of laboratory aquaria. All *P. vannamei* and the smallest sized *P. setiferus* were hatchery-reared. Because of a limited supply of laboratory-hatched *P.*

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setiferus, the two larger sizes of this species were obtained from nature. Experimental animals were blotted dry and weighed individually to the nearest 0.01 g and each size class assigned to separate experimental tanks. Within size groups the biomass of aquarium populations was similar. Some size variability between species within size groups was unavoidable due to experimental animal shortages. Each treatment was duplicated.

Table 1. Initial Sizes, Stocking Rates, and Sources of Tested Animals. Laboratory-hatched animals were artificially propagated and raised in raceways or indoor tanks. Wild shrimp were collected from Galveston Bay, Texas.

Size	*P. setiferus*	*P. vannamei*
Small	0.08±0.03 g (30)[a] Lab-hatched	0.12±0.03 g (30) Lab-hatched
Medium	0.99±0.33 g (20) Wild	1.39±0.37 g (14) Lab-hatched
Large	3.82±1.11 g (10) Wild	4.72±0.54 g (10) Lab-hatched

[a]Number of shrimp stocked in each tank shown in parentheses.

Three isonitrogenous pelletized diets (Table 2) H5G containing 5% fish protein hydrolyzate (FPH), H7.5G (7.5% FPH) and H10G (10% FPH), were prepared using the cold extrusion process with high viscosity alginate as described by Meyers and Zein-Eldin (1972). Progressive replacement of shrimp meal by FPH, rice bran, soybean meal, and cotton seed meal resulted in diets with three different animal and plant protein combinations. The amount of rice bran was adjusted to standardize the protein content of each diet at about 28%. Animal:plant (A:P) protein ratios calculated from reported or determined nutrient contents of individual ingredients were 1.5 (1.5:1) for H5G, 0.9 (0.9:1) for H7.5G, and 0.6 (1:1.5) for H10G. Gross compositions of diets were determined by biochemical analytical methods as described by Osborne and Voogt (1978).

Shrimp were fed twice daily with the pelletized diets during the 21-day experiment and uneaten food was removed each morning. Feeding more than once a day served to preserve water quality by minimizing periods of contact between uneaten food and aquarium water. It has also been shown that penaeid shrimp grow more rapidly and utilize feed more efficiently when fed more than once a day (Sedgwick 1979b).

The high quality alginate binder maintained the integrity, and thus the recognizability, of uneaten food pellets from one feeding to the next. By reducing leaching, this feature effectively protected water quality. It also permitted us to use an ad libitum feeding method in which the weight of feed was adjusted daily based on the amount remaining from the previous feedings. The regimen provided shrimp as much feed as they could consume, while minimizing excess. This procedure made it possible to conservatively estimate shrimp food consumption rates (FCR) from feeding rates, since the latter were closely tailored

to the former. This method has been found effective by others (Zein-Eldin and Meyers 1973; Sedgwick 1979a). Tanks were checked frequently during the daytime and dead shrimp and exuviae were removed, minimizing opportunities for cannibalism.

Table 2. Percentage Composition of the Test Diets

Ingredient	Diet H5G	H7.5G	H10G
Shrimp meal	31.5	18.0	5.0
Fish protein hydrolysate[a]	5.0	7.5	10.0
Soybean meal (LTI)[b]	11.0	13.0	15.0
Cottonseed meal[c]	-	4.5	9.0
Rice bran	44.0	48.5	52.5
Fish solubles	2.0	2.0	2.0
Vitamin mix[d]	2.0	2.0	2.0
Lecithin	1.0	1.0	1.0
Alginate (HV)[e]	2.5	2.5	2.5
Sodium hexametaphosphate	1.0	1.0	1.0
Chemical analysis:			
Crude protein (%)	28.0	28.5	28.3
Crude lipid (%)	6.5	7.2	6.9
Animal:plant (A:P) protein ratio	1.5:1	0.9:1	0.6:1

[a]CPSP Special "G", Sopropeche, Hamilton, Ontario, Canada.

[b]Soybean meal (Low Trypsin Inhibitor), Triple "F" Feed, Des Moines, Iowa.

[c]Glandless cottonseed meal, Rogers Delinted Cottonseed Co., Waco, Texas.

[d]Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals, Cleveland, Ohio.

[e]High viscosity sodium alginate, Kelco Co., San Diego, California.

Shrimp weight was measured again at the end of the experiment and daily growth rate and instantaneous growth rate calculated. Daily growth rate, the daily mean weight increment of the individual shrimp, was calculated from the slope of the shrimp growth regression curve. Instantaneous growth rate was used to permit comparisons of growth among groups with inherently different growth rates due to unlike animal size (Ricker 1958; Sedgwick 1979) and was defined as:

$$G = \ln (W_1/W_0)$$

where W_1 = mean final wet weight and W_0 = mean initial wet weight. Food conversion rate (FCR) and biomass increase were defined as:

FCR = Total weight of feed given/Biomass increase
Biomass increase = Final biomass — Initial biomass.

FCR was not calculated for those cases in which biomass increase was negative (Tables 3 and 4). Analysis of variance was performed to evaluate differences of both daily growth rate and instantaneous growth rate (based on a 21-day period) associated with protein type, shrimp species, and size.

Table 3. Growth, Survival and Food Conversion (FCR) of *Penaeus setiferus* after 3-Week Growth Experiment. Size classes defined in Table 1.

Shrimp size	Feed	Final wt ±s.e. (g)	Biomass gain[a] (g)	Daily growth[b] (g)	FCR[c]	Survival (%)
Large	H5G	3.81±0.94	-7.22	0.006	--	80
		4.10±0.87	-6.18		--	80
	H7.5G	3.79±1.00	-11.07	0.005	--	70
		3.96±1.08	-6.00		--	80
	H10G	3.33±0.78	-14.66	0.001	--	70
		3.83±0.64	-14.64		--	60
Medium	H5G	1.48±0.49	8.59	0.023	6.0	95
		1.43±0.47	7.76		6.2	95
	H7.5G	1.21±0.34	4.78	0.011	6.4	100
		1.26±0.32	2.92		12.0	95
	H10G	1.05±0.33	1.47	0.005	20.8	95
		1.16±0.29	0.33		80.3	95
Small	H5G	0.31±0.13	7.03	0.011	3.0	100
		0.28±0.08	5.79		3.1	97
	H7.5G	0.17±0.07	2.42	0.004	3.0	93
		0.16±0.06	1.55		4.8	83
	H10G	0.14±0.06	1.15	0.003	6.2	83
		0.14±0.06	1.54		4.9	93

[a]Biomass increase = Final biomass — Initial biomass.

[b]Daily growth rate is the slope of the regressional growth line of individual shrimp data from duplicated tanks.

[c]FCR = Total weight of feed given/Biomass increase.

RESULTS AND DISCUSSION

In the present study, diets with higher A:P ratios promoted better growth and survival of juvenile penaeids. The daily growth rate within the same size class improved 2 to 4 times when the A:P ratio of the diet was increased from 0.6 (1.5:1; H10G) to 1.5 (H5G) for both species (Tables 3 and 4). Daily growth associated with the highest (1.5) A:P ratio was significantly greater than for the other two treatments. This effect was significantly greater in the small animals than in the medium or larger-sized ones (Fig. 1). Barbieri and Cuzon (1980) found *P. japonicus* diets (protein level about 55% as compared to 28% in this study) with A:P ratio of 2 were not necessarily better than diets with A:P ratios of 1 in terms of growth. This apparent disagreement may be due to such high levels of dietary protein (55%) providing sufficient quantities of essential amino acids even though they come from inferior quality plant sources (Cowey and Sargent 1979).

The protein quality of fish feed has been demonstrated to be dependent on its level of animal protein content (Hastings 1969). Ingredients of plant origin (soybean meal and corn gluten meal) have relatively low biological value for both salmon and carp (Cowey and Sargent 1979), and usually for crustaceans (New 1976).

Table 4. Growth, Survival and Food Conversion (FCR) of *Penaeus vannamei* after 3-Week Growth Experiment. Size classes defined in Table 1.

Shrimp size	Feed	Final wt ±s.e. (g)	Biomass gain[a] (g)	Daily growth[b] (g)	FCR[c]	Survival (%)
Large	H5G	5.67±0.03	10.25	0.072	5.6	100
		5.58±1.14	8.53		6.9	100
	H7.5G	5.53±0.68	7.98	0.064	4.8	100
		5.59±0.45	8.65		4.3	100
	H10G	5.29±0.71	-5.33	0.021	--	80
		5.09±0.72	-1.71		--	90
Medium	H5G	3.36±1.00	21.11	0.120	2.8	86
		3.73±0.88	25.25		2.7	86
	H7.5G	2.55±0.73	16.41	0.077	2.5	100
		3.03±0.87	16.93		2.9	86
	H10G	2.63±0.44	9.49	0.068	4.1	79
		2.63±0.55	14.32		2.9	93
Small	H5G	0.68±0.16	16.88	0.030	2.1	100
		0.64±0.18	16.10		2.2	100
	H7.5G	0.37±0.10	6.97	0.013	2.3	97
		0.33±0.09	6.26		2.6	100
	H10G	0.28±0.08	2.81	0.010	4.8	87
		0.31±0.09	5.22		2.7	94

[a]Biomass increase = Final biomass — Initial biomass.

[b]Daily growth rate is the slope of the regressional growth line of individual shrimp data from duplicated tanks.

[c]FCR = Total weight of feed given/Biomass increase.

The performance of *P. vannamei* was better in many respects than that of *P. setiferus*. Higher growth rate ($p > F = 0.0001$), lower FCR, and lower mortality were observed in *P. vannamei* than in *P. setiferus*. Although daily growth varied with A:P ratio in the two smaller sizes of *P. vannamei*, FCR was relatively unaffected, whereas in *P. setiferus* there was a marked change in FCR associated both with A:P ratio and shrimp size classes (Tables 3 and 4). Analysis of variance of replicate instantaneous growth rates showed the effects of species, size, ratios, and the interactions of species-size and size-feed to be highly significant ($p > F = 0.001$). The fact that larger *P. vannamei* used H7.5G (A:P = 1) more efficiently than H5G (A:P = 1.5) may reflect a change from carnivorous to omnivorous or detritivorous feeding habit as the shrimp grows. *P. setiferus* did not show the same response. Cannibalism in *P. vannamei* did not cause mortality during the experiment, whereas larger wild *P. setiferus* tended to attack conspecific individuals that were molting, thus decreasing survival (to approximately 75%) and biomass. This difference in behavior may be related to the noted difference in the type of protein requirement of the two species as well as to the dissimilar histories of the larger animals (wild *P. setiferus* and laboratory-reared *P. vannamei*).

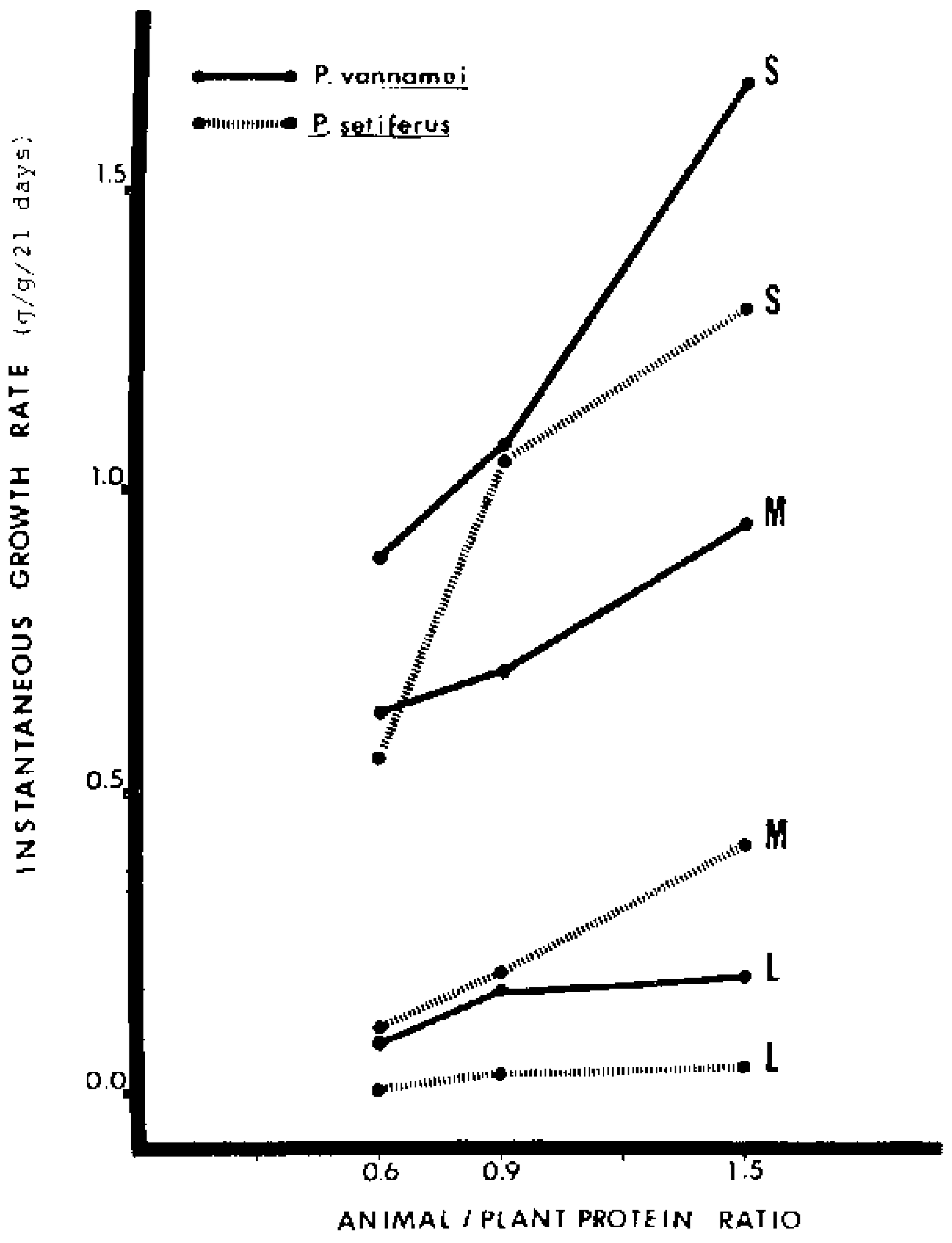

Figure 1. Instantaneous growth rates of three sizes of *Penaeus setiferus* and *P. vannamei* given 3 diets differing in animal/plant protein ratio. L = large shrimp, M = medium shrimp, S = small shrimp.

Larger juvenile *P. setiferus* (1 and 4 g) required a higher proportion of animal protein (total dietary protein = 28%) for maximum growth than did *P. vannamei*. The efficiency of food utilization, as measured by FCR, was shown to be improved for *P. setiferus* when food contained more animal protein. In *P. vannamei*, large and medium shrimp had a better FCR when fed H7.5G than H5G. Larger *P. vannamei* apparently use plant protein as efficiently as animal protein. This result may imply an advantage for *P. vannamei* over *P. setiferus* in terms of variety and cost of culture diets.

Instantaneous growth rate declined proportionally as the shrimp size increased (Fig. 1). Hysmith et al. (1972) pointed out that the need for raw materials for body synthesis is reduced as shrimp grow larger. Results from the present study may indicate that a diet high in animal protein supplies amino acids more effective for tissue synthesis and growth while plant protein can furnish the nutritional requirement for maintenance. In this respect, an animal-protein-rich diet gives shrimp a combination of amino acids which is easier to assimilate, thus enhancing growth. Diets with amino acid profiles similar to that of short-necked clam (*Tapes*) flesh have proved to be very effective for the growth of *P. japonicus* (Deshimaru and Shigueno 1972).

Maximum growth can be achieved more cost-effectively with a progressive reduction of dietary protein levels as young shrimp grow (Colvin and Brand 1977). The present results suggest that progressive replacement of animal protein with plant protein may also be justified as juvenile growth proceeds. A series of feeds with different protein levels and qualities appears to be necessary to maximize growth at minimum cost for different life-cycle stages of penaeid shrimp. The A:P ratio may be a useful measure to include in the evaluation of shrimp diets. Studies of larger animals, approaching sexual maturity, may reveal changes in shrimp growth responses to dietary A:P ratios. Further research is needed to test additional sources and combinations of animal and plant proteins and larger sizes of penaeid shrimp.

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